

Jungblut
Prelim. Amd.U.S. Nat. Phase
PCT/DE98/01499
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In claim 5, lines 1 and 2, please delete "one of the claims 1 to 3" and substitute therefor: -- claim 1 --.

In claim 7, line 1, please delete "one of the claims 1 to 3" and substitute therefor: -- claim 1 --

In claim 11, line 1, please delete "one of the claims 1 to 3" and substitute therefor: -- claim 1 --.

In claim 12, line 1, please delete "one of the claims 1 to 3" and substitute therefor: -- claim 1 --.

Please Add the Following New Claims:

13. Monoclonal antibodies according to claim 2, with the hybridoma cells enabled to produce monoclonal human-CD28 specific animal antibodies being available through

a) creation of a plasmid by means of insertion of human-CD28 cDNA into the pH β APr-1-neo vector following excision of the Sall-HindIII fragment and production of protoplasts from Escherichia coli (MC 1061) with carry the plasmid,

b) fusing the protoplasts with mouse A20J and/or L929 tumor cells by means of polyethylene glycol,

c) cultivation of the transfected cells received in phase b),

d) screening of the transfected mouse A20J and/or L929 cells for the expression of human CD28 and selection of mouse A20J and/or L929 cells expressing human-CD28,

e) immunization of BALB/c mice with mouse A20J and or L929 cells expressing human-CD28,

f) removal of spleen cells of the mice immunized in this way and fusing the spleen cells with cells of the cell line X63-Ag 8.653 by means of polyethylene glycol,

g) selection of the hybridoma cells received in this way with the condition that in the supernatant of selected hybridoma cells there are antibodies contained which bind on human CD28 expressing mouse A20J and/or L929 cells and

h) cultivation/sub-cloning of the selected hybridoma cells obtained in phase g).

14. Hybridoma cells for the production of monoclonal antibodies according to claim 2 which are available through the following procedural steps:

a) creation of a plasmid by means of insertion of human-CD28 cDNA into the pH β APr-1-neo vector following excision of the Sall-HindIII fragment and production of protoplasts from Escherichia coli (MC 1061) with carry the plasmid,

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- b) fusing the protoplasts with mouse A20J and/or L929 tumor cells by means of polyethylene glycol,
c) cultivation of the transfected cells received in phase b),
d) screening of the transfected mouse A20J and/or L929 cells for the expression of human CD28 and selection of mouse A20J and/or L929 cells expressing human-CD28,
e) immunization of BALB/c mice with mouse A20J and or L929 cells expressing human-CD28,
f) removal of spleen cells of the mice immunized in this way and fusing the spleen cells with cells of the cell line X63-Ag 8.653 by means of polyethylene glycol,
g) selection of the hybridoma cells received in this way with the condition that in the supernatant of selected hybridoma cells there are antibodies contained which bind on human CD28 expressing mouse A20J and/or L929 cells.

15. Hybridoma cells for the production of monoclonal antibodies according to claim 3 which are available through the following procedural steps:

- a) creation of a plasmid by means of insertion of human-CD28 cDNA into the pH β APr-1-neo vector following excision of the Sall-HindIII fragment and production of protoplasts from Escherichia coli (MC 1061) with carry the plasmid,
b) fusing the protoplasts with mouse A20J and/or L929 tumor cells by means of polyethylene glycol,
c) cultivation of the transfected cells received in phase b),
d) screening of the transfected mouse A20J and/or L929 cells for the expression of human CD28 and selection of mouse A20J and/or L929 cells expressing human-CD28,
e) immunization of BALB/c mice with mouse A20J and or L929 cells expressing human-CD28,
f) removal of spleen cells of the mice immunized in this way and fusing the spleen cells with cells of the cell line X63-Ag 8.653 by means of polyethylene glycol,
g) selection of the hybridoma cells received in this way with the condition that in the supernatant of selected hybridoma cells there are antibodies contained which bind on human CD28 expressing mouse A20J and/or L929 cells.

16. Procedure for the production of monoclonal antibodies according to claim 2 with the following procedural steps:

- a) production of hybridoma cells enabled to produce monoclonal human-CD28 specific animal antibodies by means of an immunization with non-T tumor cell lines on which human-CD28 is expressed,
b) if applicable, humanization of the monoclonal animal antibodies available from the hybridoma cells pursuant to phase a) through a biochemical or gene-technological exchange of constant components of the animal antibodies against analogous constant components of a human antibody or replacement of genes or the hybridoma cells corresponding to the components;

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c) secreting of the antibody in hybridoma cell cultures and isolation of the antibodies from it or production of the antibodies by injection of the hybridoma cells into animals, for example, mice, and isolation of the antibodies from the body fluid of the animal.

17. Procedure for the production of monoclonal antibodies according to claim 3 with the following procedural steps:

a) production of hybridoma cells enabled to produce monoclonal human-CD28 specific animal antibodies by means of an immunization with non-T tumor cell lines on which human-CD28 is expressed,

b) if applicable, humanization of the monoclonal animal antibodies available from the hybridoma cells pursuant to phase a) through a biochemical or gene-technological exchange of constant components of the animal antibodies against analogous constant components of a human antibody or replacement of genes or the hybridoma cells corresponding to the components;

c) secreting of the antibody in hybridoma cell cultures and isolation of the antibodies from it or production of the antibodies by injection of the hybridoma cells into animals, for example, mice, and isolation of the antibodies from the body fluid of the animal.

18. Use of monoclonal antibodies according to claim 2 for the production of a medicine for the therapeutic treatment of the human body.

19. Use of monoclonal antibodies according to claim 3 for the production of a medicine for the therapeutic treatment of the human body.

20. Use of monoclonal antibodies according to claim 2 for the treatment of diseases of the human body.

21. Use of monoclonal antibodies according to claim 3 for the treatment of diseases of the human body.

22. Procedures for the therapeutic treatment of the human body with monoclonal antibodies according to claim 2 being used.

23. Procedures for the therapeutic treatment of the human body with monoclonal antibodies according to claim 3 being used.

24. Hybridoma cells for the production of monoclonal antibodies according to claim 13 which are available through the following procedural steps:

a) creation of a plasmid by means of insertion of human-CD28 cDNA into the pH β APr-1-neo vector following excision of the Sall-HindIII fragment and production of protoplasts from Escherichia coli (MC 1061) with carry the plasmid,

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- b) fusing the protoplasts with mouse A20J and/or L929 tumor cells by means of polyethylene glycol,
 - c) cultivation of the transfected cells received in phase b),
 - d) screening of the transfected mouse A20J and/or L929 cells for the expression of human CD28 and selection of mouse A20J and/or L929 cells expressing human-CD28,
 - e) immunization of BALB/c mice with mouse A20J and or L929 cells expressing human-CD28,
 - f) removal of spleen cells of the mice immunized in this way and fusing the spleen cells with cells of the cell line X63-Ag 8.653 by means of polyethylene glycol,
 - g) selection of the hybridoma cells received in this way with the condition that in the supernatant of selected hybridoma cells there are antibodies contained which bind on human CD28 expressing mouse A20J and/or L929 cells.

25. Procedure for the production of monoclonal antibodies according to claim 13 with the following procedural steps:

a) production of hybridoma cells enabled to produce monoclonal human-CD28 specific animal antibodies by means of an immunization with non-T tumor cell lines on which human-CD28 is expressed,

b) if applicable, humanization of the monoclonal animal antibodies available from the hybridoma cells pursuant to phase a) through a biochemical or gene-technological exchange of constant components of the animal antibodies against analogous constant components of a human antibody or replacement of genes or the hybridoma cells corresponding to the components;

c) secreting of the antibody in hybridoma cell cultures and isolation of the antibodies from it or production of the antibodies by injection of the hybridoma cells into animals, for example, mice, and isolation of the antibodies from the body fluid of the animal.

26. Use of monoclonal antibodies according to claim 13 for the production of a medicine for the therapeutic treatment of the human body.

27. Use of monoclonal antibodies according to claim 13 for the treatment of diseases of the human body.

28. Procedures for the therapeutic treatment of the human body with monoclonal antibodies according to claim 13 being used.

29. Procedure according to claim 16, with the hybridoma cells enabled to produce monoclonal human-CD28 specific animal antibodies being produced in the following procedural steps:

a) creation of a plasmid by means of insertion of human-CD28 cDNA into the pH β APr-1-neo vector following excision of the Sall-HindIII fragment and production of protoplasts from Escherichia coli (MC1061) which carry the plasmid,

- b) fusing of the protoplasts with mouse A20J and/or L929 tumor cells by means of polyethylene glycol,
- c) cultivation of the transfected cells received in phase b),
- d) screening of the transfected mouse A20J and /or L929 cells for the expression of human-CD28 and selection of mouse A20J and/or L929 cells expressing human-CD28,
- e) immunization of BALB/c mice with mouse A20J and/or L929 cells expressing human-CD28,
- f) removal of spleen cells of the mice immunized in this way and fusing the spleen cells with cells of the cell line X63-Ag 8.653 by means of polyethylene glycol and
- g) selection of the hybridoma cells received in this way with the condition that in the supernatant of selected hybridoma cells there are antibodies contained which bind on human-CD28 expressing mouse A20J and/or L929 cells.

30. Procedure according to claim 17, with the hybridoma cells enabled to produce monoclonal human-CD28 specific animal antibodies being produced in the following procedural steps:

- a) creation of a plasmid by means of insertion of human-CD28 cDNA into the pH β APr-1-neo vector following excision of the Sall-HindIII fragment and production of protoplasts from Escherichia coli (MC1061) which carry the plasmid,
- b) fusing of the protoplasts with mouse A20J and/or L929 tumor cells by means of polyethylene glycol,
- c) cultivation of the transfected cells received in phase b),
- d) screening of the transfected mouse A20J and /or L929 cells for the expression of human-CD28 and selection of mouse A20J and/or L929 cells expressing human-CD28,
- e) immunization of BALB/c mice with mouse A20J and/or L929 cells expressing human-CD28,
- f) removal of spleen cells of the mice immunized in this way and fusing the spleen cells with cells of the cell line X63-Ag 8.653 by means of polyethylene glycol and
- g) selection of the hybridoma cells received in this way with the condition that in the supernatant of selected hybridoma cells there are antibodies contained which bind on human-CD28 expressing mouse A20J and/or L929 cells.

31. Procedure according to claim 25, with the hybridoma cells enabled to produce monoclonal human-CD28 specific animal antibodies being produced in the following procedural steps:

- a) creation of a plasmid by means of insertion of human-CD28 cDNA into the pH β APr-1-neo vector following excision of the Sall-HindIII fragment and production of protoplasts from Escherichia coli (MC1061) which carry the plasmid,
- b) fusing of the protoplasts with mouse A20J and/or L929 tumor cells by means of polyethylene glycol,
- c) cultivation of the transfected cells received in phase b),
- d) screening of the transfected mouse A20J and /or L929 cells for the expression of human-CD28 and selection of mouse A20J and/or L929 cells expressing human-CD28,

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- e) immunization of BALB/c mice with mouse A20J and/or L929 cells expressing human-CD28,
- f) removal of spleen cells of the mice immunized in this way and fusing the spleen cells with cells of the cell line X63-Ag 8.653 by means of polyethylene glycol and
- g) selection of the hybridoma cells received in this way with the condition that in the supernatant of selected hybridoma cells there are antibodies contained which bind on human-CD28 expressing mouse A20J and/or L929 cells.

32. Use according to claim 18 for the production of a medicine for the treatment of diseases with pathologically reduced numbers of CD4 T cells, in particular AIDS or following stem cell transplantations after chemotherapy of leukemic diseases.

33. Use according to claim 19 for the production of a medicine for the treatment of diseases with pathologically reduced numbers of CD4 T cells, in particular AIDS or following stem cell transplantations after chemotherapy of leukemic diseases.

34. Use according to claim 26 for the production of a medicine for the treatment of diseases with pathologically reduced numbers of CD4 T cells, in particular AIDS or following stem cell transplantations after chemotherapy of leukemic diseases.

35. Use according to claim 18 for the production of a medicine for the potentiation and/or qualitative influencing of immune reactions in protective inoculations.

36. Use according to claim 19 for the production of a medicine for the potentiation and/or qualitative influencing of immune reactions in protective inoculations.

37. Use according to claim 26 for the production of a medicine for the potentiation and/or qualitative influencing of immune reactions in protective inoculations.

38. Use according to claim 18 for the production of a medicine to influence the quality of the T cell reaction; in particular to influence the production of various effector molecules, for example cytokines and chemokines and their receptors, for example in auto-immune diseases and AIDS.

39. Use according to claim 19 for the production of a medicine to influence the quality of the T cell reaction; in particular to influence the production of various effector molecules, for example cytokines and chemokines and their receptors, for example in auto-immune diseases and AIDS.

40. Use according to claim 26 for the production of a medicine to influence the quality of the T cell reaction; in particular to influence the production of various effector molecules, for example cytokines and chemokines and their receptors, for example in auto-immune diseases and AIDS.